REMARKS

Applicant respectfully requests reconsideration. Claims 1-73 were previously pending in this application. Claims 9, 10, 13, 15-17, 19, 22-38 and 41-73 were withdrawn. Claims 1-8, 11, 12, 14-16, 18, 20, 21, 39 and 40 were examined.

Applicant has amended claim 15 to depend from claim 8.

No new matter has been added.

Objections to the Specification

1. The abstract of the disclosure is objected to because it does not commence on a sheet separate from other materials of the disclosure.

Applicant has amended the specification to provide the abstract on a separate sheet following the claims, and respectfully requests reconsideration and withdrawal of the objection.

2. The Examiner indicated that there are sequence disclosures on page 36 of the specification that are not present in the Sequence Listing and/or are not identified by sequence identifier numbers.

Applicant respectfully requests reconsideration and withdrawal of the objection on the basis that the sequence identifiers (SEQ ID NOs 5 to 8) in fact are present on page 36 (see lines 14, 19, 23 and 28) and these sequences in fact are present in the Sequence Listing.

Claims Objections

Claims 3, 4 and 18 are objected to under 37 C.F.R. §1.75(c) as being of improper dependent form for failing to further limit the subject matter of a previous claim. Applicant respectfully disagrees and requests reconsideration of the objection.

Claim 1 reads:

1. A genetically modified non-human mammal or cell characterised in that it does not comprise a nucleic acid sequence which itself encodes any endogenous immunoglobulin heavy chain constant region locus polypeptide and in that one or more endogenous Ig H Variable region, one or more endogenous Ig H D segment, and one or more endogenous Ig H J segment nucleic acid sequences are present.

Claim 3 reads:

3. A genetically modified non-human mammal or cell according to claim 1 characterised in that it does not comprise a nucleic acid sequence which itself encodes any immunoglobulin heavy chain constant region (Ig HC) polypeptide.

Thus, according to claim 1, no nucleic acid sequence that encodes any ***endogenous*** heavy chain constant region locus is present; however, nucleic acid sequence that encodes an exogenous heavy chain constant region locus polypeptide could be present, whereas according to claim 3, no nucleic acid sequence that encodes any heavy chain constant region polypeptide, ***endogenous or exogenous***, is present.

Thus, the subject matter of claim 3 is limited relative to claim 1.

Claim 4 reads:

4. A genetically modified non-human mammal or cell according to claim 1 characterised in that all immunoglobulin heavy chain constant region gene sequences are absent or partially absent from the genome.

According to claim 1, the genetically-modified non-human mammal or cell does not comprise nucleic acid sequence that encodes any endogenous heavy chain region locus polypeptide; as explained at paragraph 23: this can be achieved by targeted deletion of all or essentially all endogenous IgH C gene sequences. The deletion can be of all endogenous Ig HC region genes and intervening sequences (complete exon/intron removal or clean deletion) or essentially all endogenous Ig HC sequences by deletion of an extensive part of the endogenous Ig HC region gene sequence such that expression of any of the Ig HC genes is prevented.

Thus, the subject matter of claim 4 is limited relative to claim 1.

Claim 18 is not directly dependent on claim 1; it depends on claim 16, which specifies that the genetically modified non-human mammal is a mouse:

16. A genetically modified non-human mammal according to claim 1 characterised in that it is a mouse.

Claim 18 specifies the endogenous mouse genes that are absent:

18. A genetically modified mouse according to claim 16, or a genetically modified mouse cell according to claim 17, characterised in that all eight endogenous Ig HC genes μ, δ, γ3, γ1, γ2a, γ2b, ε and α are absent or partially absent.

Thus the subject matter of claim 18 is further limited relative to claim 1.

Accordingly, Applicant respectfully requests that the objection to the claims be withdrawn.

Rejections Under 35 U.S.C. § 102/103

Claims 1-6, 8, 11, 12, 14-16, 18, 20, 21, 39 and 40 are rejected under 35 U.S.C. § 102(a) as being anticipated by or, in the alternative, are rejected under 35 U.S.C. § 103(a) as being obvious over Rajewsky et al. (US 6,570,061). Applicant respectfully traverses the rejection.

Rajewsky *et al.* disclose methods for targeted replacement of individual gene segments with a gene or gene segment of another species. Rajewsky *et al.* state (column 2, lines 28 to 32) that:

> "...the task of the present invention was to make available a directly successful method in one step for production of genetically engineered non-human mammals that contain homologous gene segments from other mammals via homologous recombination."

At column 5, lines 54 to 67, Rajewsky *et al.* state:

> "The subject transgenic animals have a native immunoglobulin (Ig) constant region gene functionally replaced with a human constant region gene, that is, the human constant region segment replaces the native gene segment in the genetic recombination and expression events associated with an antibody response. The native gene may be deleted or inactivated. The constant region gene is herein defined as the constant region exons, and optionally including introns, encoding the secreted portion of a mature immunoglobulin chain. In a preferred embodiment, the host transmembrane and cytoplasmic portion will be retained. An intact switch region, either human or from the native gene, will be present at the heavy chain locus."

Thus Rajewsky *et al.* focus on ***replacement*** of an endogenous gene with a human gene.

Accordingly, Rajewsky *et al.* do **not** disclose genetically modified mice or cells in accordance with the present invention, which do not encode ***any*** Ig HC region locus polypeptide (claim 3), since the strategy described in Rajewsky requires replacement of an endogenous gene with a human gene.

Rajewsky *et al.* state at column 6:

> "For most applications it is desirable to have the genes for both the Ig heavy and light chains constant regions replaced with the human genes".

However, Rajewsky *et al.* do not provide an enabling disclosure of a genetically modified non-human mammal or cell that does not does not comprise a nucleic acid sequence which itself encodes ***any endogenous*** Ig HC locus polypeptide (claims 1 and 2).

At the time of filing of the invention, those skilled in the art would not have expected that the methodology in Rajewsky *et al.* could be applied to achieve silencing of the entire endogenous Ig HC locus by deletion. The short constructs employed by Rajewsky *et al.* for replacement of the murine γ1 gene with the human γ1 gene could not be used to achieve deletion (or replacement) of the entire Ig HC locus. The method described in Rajewsky *et al.* involves use of a single short construct which is introduced into a mouse ES cell and integrated into the target murine γ1 gene in a single homologous recombination event; following transient expression of Cre recombinase in the ES cell, the introduced neo^r-HSVtk and murine gene sequence γ1 flanked by loxP sites is deleted, leaving the introduced human γ1 gene sequence inserted into the murine genome.

The method employed by Applicant to achieve deletion of the entire Ig HC locus (all eight endogenous Ig HC genes μ, δ, γ3, γ1, γ2a, γ2b, ε and α) required integration of two different targeting constructs (each carrying a loxP site) into the mouse genome. For successful Ig HC locus deletion, the targeting constructs had to be integrated at either end of the Ig HC locus on a single allele (tandem allele integration). Following successful generation of a mouse with both of the targeted integrations on the same allele, the mouse was bred with a Cre expressing mouse to obtain progeny in which the Ig HC locus flanked by the loxP sequences was deleted.

The techniques described in Rajewsky *et al.* could not be used to achieve deletion of the Ig HC locus. Gene targeting requires that two major hurdles be overcome: homologous recombination and germline transmission. Gene targeting frequencies are extremely variable and not all of the parameters that affect the frequency of homologous recombination and germ line transmission are known. High homology between the target sequence and targeting vector is required and the frequency of homologous recombination depends both on the degree of sequence match and the length of the matching sequences. Small sequence divergences can exert a disproportionately large negative effect on the frequency of homologous recombination. The Ig HC locus contains many repeated sequences and these present difficulties in accurate integration of a targeting construct in the desired location. It is recognized in the art that there is

a practical limit to the amount of DNA that can be deleted by gene targeting and that gene targeting frequency decreases with as the length of the desired deletion increases.

Rajewsky *et al.* does not teach or suggest how deletion of the entire Ig HC region could be achieved, nor that the Cre-loxP method used would be effective to achieve such a large deletion. Effectively, Rajewsky *et al.* teaches only production of immunoglobulins in a host background which includes endogenous heavy chain constant region genes. Using the mice described by Rajewsky *et al.*, it would not be possible to generate immunoglobulins with only an exogenous constant region or regions.

Rajewsky *et al.* exemplify only the replacement of a single murine γ1 gene with a single human γ1 gene; thus in the modified mice exemplified, the other murine heavy chain genes (μ, δ, γ3, γ2a, γ2b, ε and α) are still present in the mouse genome and were expressed (see Figure 5). Accordingly Rajewsky *et al.* do not disclose genetically modified non-human animals or cells in accordance with the present invention which do not encode ***any endogenous*** Ig HC region locus polypeptide (claim 1), or ***any*** Ig HC region locus polypeptide (claim 3).

In Rajewsky *et al.*, it is preferred that transgenic animals are obtained that undergo affinity maturation and a class switch from the native immunoglobulin to a humanized form (column 3, paragraph 2). To do this, Rajewsky *et al.* requires that at least the endogenous Ig HC μ region must be expressed.

From column 5 of Rajewsky *et al.*:

> "Transgenic animals are obtained that produce high affinity antibodies with human constant region sequences. During in vivo affinity maturation animals with a native Cμ region are able to class switch to a transgenic C region, e.g. Cγ, Cα, Cδ or Cε."

From column 6:

> "At the host heavy chain locus, at least one of the isotypes will be functionally replaced, e.g. Cμ , Cγ, Cα, Cδ or Cε. The transgenic human gene may be the counterpart to the native gene, e.g. Cγ1 → Cγ1. Preferably the replaced host region will be other then Cμ."

Accordingly Rajewsky *et al.* do **not** disclose genetically modified mice or cells in accordance with the present invention which do not encode ***any endogenous*** Ig HC region locus polypeptide (claims 1 and 2), or ***any*** Ig HC region locus polypeptide (claim 3).

Until the present invention, no one had succeeded in making an animal or cell retaining IgH, V, D and J sequences yet lacking all endogenous Ig HC constant region gene sequences to such an extent that no endogenous functional heavy chain gene product could be expressed. Indeed, until the production of the Ig HC locus knock-out of the present invention it was uncertain that this phenotype could be generated, since it was believed that "trans" recombination between endogenous V D J region sequences and constant region-like genes (e.g. T cell receptor loci, or pseudogenes) could lead effectively to reconstitution of endogenous constant region function.

Therefore, claims 1-6, 8, 11, 12, 14-16, 18, 20, 21, 39 and 40 are neither anticipated by nor obvious over Rajewsky *et al.* (US 6,570,061). Accordingly, Applicant respectfully requests that the rejection of the claims be withdrawn.

Rejections Under 35 U.S.C. § 103

Claims 1 and 7 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Rajewsky *et al.* (US 6,570,061) in view of Fell *et al.* (US 5,202,238). Applicant respectfully traverses the rejection.

Rajewsky *et al.* teach generation of mice that express the human kappa gene using a construct that includes a light chain kappa intron enhancer to replace the murine kappa gene (MCκ) with the human kappa gene (HCκ).

Separately, Rajewsky teaches generation of mice that express the human IgG1 gene (and also murine IgM, IgD, IgG3, IgG2b, IgG2a IgE and IgA) using a construct for replacement of the murine γ1 gene with a human γ1 gene.

Fell fails to remedy the deficiencies in the teaching of Rajewsky. Fell teaches replacement of a single murine Ig gene with a single gene encoding the constant region of human IgG1 using a targeting vector that includes Ig HC enhancer sequence.

Thus the combination of Rajewsky *et al.* and Fell *et al.* fails to describe deletion to produce genetically modified non-human animals or cells in accordance with the present invention which do not encode ***any endogenous*** Ig HC region locus polypeptide. According to the combination of Rajewsky *et al.* and Fell *et al.*, mice are produced which express a single human Ig gene in a background wherein the remaining endogenous Ig H C region genes are retained and expressed. Thus the mice of Rajewsky and Fell will produce mixed antibodies with not only the introduced human IgG1 heavy chain, but also murine Ig heavy chains.

Accordingly, Applicant respectfully requests that the rejection of the claims as obvious over the combination of Rajewsky *et al.* and Fell *et al.* be withdrawn.

Rejections Under 35 U.S.C. § 112, Second Paragraph

Claim 15 is rejected under 35 U.S.C. § 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which Applicant regards as the invention.

Applicant has amended claim 15 to depend from claim 8. Accordingly, the rejection of claim 15 for lack of antecedent basis is moot.

Rejections Under 35 U.S.C. § 112, First Paragraph

Claims 1-8, 11, 12, 14-16, 18, 20, 21, 39 and 40 are rejected under 35 U.S.C. §112, first paragraph, because the specification, while being enabling for making a genetically modified

mouse lacking endogenous IgH constant region and containing the endogenous IgH VDJ region, allegedly does not reasonably provide enablement for making any non-human mammal having the recited features and it does not reasonably provide enablement for how to use the claimed invention. Applicant respectfully traverses the rejection.

First, the Examiner cites Table 1 as evidence that deletion of endogenous the Ig HC was "lost" in the second generation as no progeny of the Ig HC deletion mice were obtained. Office Action at page 8.

This is not correct, as can be seen from Table 1, which shows that in the second generation 4 mice (11%) had a locus deletion. See the third row of the rightmost column of Table 1.

Second, the Examiner alleges that the state of the art supports only mouse ES cells for use in production of transgenic mice/gene knock out mice. Office Action at pages 8-9. The Examiner cites Moreadith *et al.* (1997) as evidence, but Moreadith *et al.* was published seven years before the (international) filing date of the present application. Thus Moreadith *et al.* is not representative of the state of the art at the filing date of the present invention.

In fact, Moreadith *et al.* report (Summary, page 214) the following:

> "The advent of techniques to generate gain-of-function and loss-of-function mutation in laboratory animals represents one of the main accomplishments in cell and molecular biology in mammals over the past two decades. Although the technology is generally limited only to the mouse at present, substantial effort is underway to develop these techniques, and to refine existing techniques, in other species, Putative pluripotential ES cell lines have been derived in a number of other species including Hamster [70], pig [71 – 75], sheep [73], cattle [76], rabbit, [77], rat [78], mink [79], monkey [80] and even humans [81]. Thus it seems likely the technology will be advanced into these additional species over the next few years, ..." (emphasis added)

Thus, Moreadith *et al.* in 1997 presage developments in the field beyond manipulation of mouse ES cells.

Third, the Examiner argues that "putative" ES cells have not been demonstrated to give rise to germline tissue or the whole animal. Office Action at pages 8-9. Applicant submits that this is not correct.

Pera *et al.* (2000, cited by the Examiner) relates to generation of human ES cells, which are outside the scope of the present invention. The Examiner cites page 6, second column:

> "Thus far only mouse EG or ES cells meet these generic criteria. Primate ES cells meet the first three of four criteria, but not the last. Numerous other candidate mammalian ES cells have been described over the years in domestic and laboratory species, but only in the mouse have all criteria been met rigorously."

The four criteria are recited in the table given at page 6, second column. The last criterion listed is: "clonally derived cultures capable of spontaneous differentiation into extra embryonic tissue and somatic cells representative of all three germ layers in teratomas or in vitro". The primate cells described at page 6, column 2, paragraph 2 of Pera *et al.* would appear to at least partially meet the fourth criterion. Applicant submits that it is not necessary that candidate mammalian ES calls meet the criteria in the table rigorously. They can be used in methods described in the present invention.

Kuroiwa *et al.* (2004, cited by the Examiner), which published shortly after the filing date of the (international) application, describes methods for sequential gene targeting in primary fibroblast cells and Cre-loxP mediated deletion for generation of calves heterozygous and homozygous for knock out of the transcriptionally silent gene Ig-mu. Kuroiwa *et al.* demonstrates that gene targeting and germline transmission can be achieved without the use of ES cells.

Germline competent ES cells have been reported in rat (Buehr *et al.* (2008); Li *et al.* (2008)) and methods for gene targeting have been described in cattle (Richt *et al.* (2007)); copies of the papers are provided herewith. Richt *et al.* refers to the method of Kuroiwa *et al.* (2004) cited by the Examiner. Thus as a result of routine developments in the art and without undue experimentation, those skilled in the art are able to perform genetic manipulation of ES cells, ES

cell lines or other pluripotent cells from species other than mice and Applicant submits that the claimed invention should be considered to be enabled for the full the scope of the claims.

Fourth, the Examiner argues that the specification fails to teach that, following introduction of exogenous Ig genes, animals/cells with endogenous Ig HC locus deletion would be able to express foreign Ig. Office Action at pages 9-10. Applicant respectfully submits that this is not the case.

As can be seen at pages 555 to 556 of the enclosed review (Brüggemann, M. (2004). Human Monoclonal Antibodies from Translocus Mice. Molecular Biology of B Cells. Eds Alt, Honjo and Neuberger. Elsevier. pp 547-561), human Ig loci have been expressed in a background in which the endogenous mouse H and κ L chain loci have been rendered nonfunctional. Brüggemann states that the block in B-cell development "can be overcome by the introduction of an IgH translocus, which kick-starts B-cell development and leads to (human) antibody production." See page 556, left column.

Thus, practicing the claimed invention would not require undue experimentation for the person of skill in the art, given the level of skill and knowledge in the art, the nature of the invention, and the guidance (including working examples) provided in the specification. Accordingly, Applicant submits that the claimed invention is enabled, and respectfully requests that the Examiner withdraw the rejection of the claims.

CONCLUSION

A Notice of Allowance is respectfully requested. The Examiner is requested to call the undersigned at the telephone number listed below if this communication does not place the case in condition for allowance.

If this response is not considered timely filed and if a request for an extension of time is otherwise absent, Applicant hereby requests any necessary extension of time. If there is a fee occasioned by this response, including an extension fee, that is not covered by an enclosed check, please charge any deficiency to Deposit Account No. 23/2825.

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